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(54) Title: BENZAZOLE COMPOUNDS FOR USE IN THERAPY

(57) Abstract

There are disclosed herein benzazole compounds, exemplified by 2-(4-aminophenyl)benzothiazole and analogues or salts thereof, which exhibit very significant selective cytotoxic activity in respect of tumour cells, especially breast cancer cells, and which provide potentially useful chemotherapeutic agents for treatment of breast cancer.

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Benzazole compounds for use in therapy

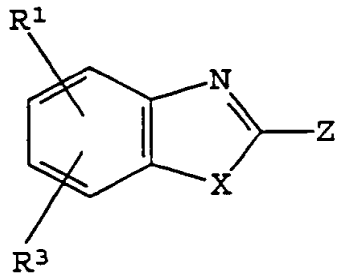
Field of the Invention

The present invention relates to certain biologically active benzazole compounds, particularly benzothiazole and benzoxazole compounds, which are able selectively to inhibit proliferation of certain mammalian tumour cells, particularly breast cancer cells, and which are accordingly of interest for use as active chemotherapeutic agents in antitumour therapy, especially in connection with the treatment of breast cancer.

Summary of the Invention

In one aspect this invention provides, for use in therapy, 2-arylbenzazole compounds that are especially active in inhibiting proliferation of certain breast cancer tumour cells, said compounds being exemplified by 2-(4-aminophenyl)benzothiazole and close analogues or acid addition salts thereof.

More specifically, the 2-arylbenzazole compounds of the present invention are generally compounds having the structural formula I,



I

characterised in that

X is S or O;

R^1 and R^3 are each independently hydrogen, alkyl, hydroxyl, alkoxy or aralkoxy

Z is aryl;

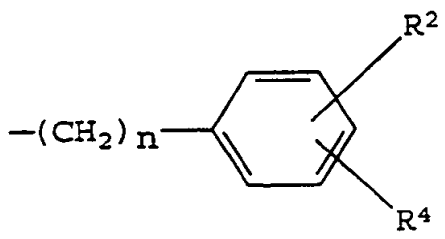
subject to the proviso that alkyl groups when present as such in the compound or as a moiety in other groups such as alkoxy are each composed of less than 6 carbon atoms;

or are compounds that are pharmaceutically acceptable salts of compounds represented by the structural formula specified above.

In addition to phenyl or substituted phenyl aromatic groups, the term aryl as used herein may also include arylalkyl (aralkyl) or aromatic heterocyclic groups, particularly nitrogen-containing heterocyclic groups such as pyridyl. Arylalkyl groups can include groups such as benzyl or cinnamyl for example, and in general the aryl groups will contain at least one nitrogen atom or nitrogen containing substituent, especially an amine or a group readily convertible into an amine.

The invention also includes compounds that in use can act as precursors or prodrugs and readily break down or be converted, for example by metabolic processes, in the animal body or other biological systems so as to form the particular biologically active compounds hereinbefore specified, especially 2-arylbenzazole compounds in which the aryl group has an amino group substituent. The claims appended hereto should therefore be construed accordingly in determining the scope of the invention.

In at least most preferred embodiments of the invention wherein the benzazole compound is as specified above, the group Z may be represented by the structural formula



II

where n = 0 or 1;

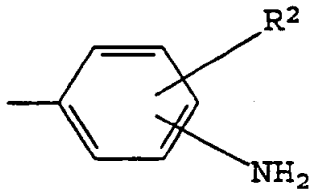
R^2 is hydrogen, NH_2, NO_2, N_3, halogen or an alkyl or substituted alkyl oxysulphonyl group;

R^4 is hydrogen, NO_2, N_3, pyrrolidino, piperidino, morpholino or NR^5R^6 where

R^5 and R^6 each represent hydrogen or alkyl;

with the further proviso that R^2 and R^4 are not both hydrogen. Also, at least one of R^2 and R^4 will generally be an amino or a substituted amino group, or some other nitrogen-containing group convertible into an amino group. Furthermore, usually, but not necessarily, R^4 will be para or in the 4-position of the phenyl group.

Particularly preferred embodiments include at least some compounds in which Z can be represented by the structural formula



III

where R^2 is as previously defined. If R^2 is halogen, often it will preferably be a 2-Cl group.

In some compounds within the scope of the invention, it is also possible for the group $-(CH_2)_n-$ in the above structure II to be replaced by an alkenylic group such as -CH=CH-

Preferred compounds of the invention in accordance with formula I wherein R^3 is hydrogen include compounds in

which R^1 is alkoxy or benzyloxy, and X is preferably sulphur. More generally, preferred compounds of the invention in accordance with the structural formula I may be further characterised by at least one of the following features:

(a) at least some alkyl groups when present as such or as a moiety in other groups such as alkoxy are methyl or ethyl;

(b) halo substituents, when present, are selected from fluorine and chlorine;

(c) R^2 is hydrogen or 2-halogen, and R^4 is amino.

Compounds in accordance with the invention which conform to formula I wherein Z has the structural formula II with n=0, and which are of particular interest, include those compounds where the combination of substituents R^1, R^2, R^3, R^4 and X is selected from the following combinations:

R^1	R^3	X	R^2	R^4
H	H	S	H	4-NH_2
H	H	S	3-NH_2	4-H
H	H	S	2-NH_2	4-H
H	H	S	H	4-NMe_2
H	H	S	H	4-NEt_2
H	H	S	H	4-Pyrrolidino
H	H	S	H	4-Piperidino
H	H	S	H	4-Morpholino
H	H	S	H	4-NO^2
H	H	S	3-NO_2	4-H
H	H	S	2-NO_2	4-H
H	H	S	H	4-N_3
H	H	S	3-N_3	4-H
H	H	S	2-N_3	4-H
H	H	S	3-I	4-NH_2
H	H	O	H	4-NH_2

H	H	O	H	4-N_3
H	H	S	2-F	4-NH_2
H	H	S	2-F	4-NO_2
H	H	S	2-F	4-N_3
H	H	S	2-Cl	4-NH_2
H	H	S	2-Cl	4-NO_2
H	H	S	2-Cl	4-N_3
4-OMe	H	S	2-Cl	4-NO_2
5-OMe	H	S	2-Cl	4-NO_2
6-OMe	H	S	H	4-NO_2
6-OMe	H	S	2-Cl	4-NO_2
7-OMe	H	S	2-Cl	4-NO_2
5-OBenzyl	H	S	2-Cl	4-NO_2
6-OBenzyl	H	S	2-Cl	4-NO_2
7-OBenzyl	H	S	2-Cl	4-NO_2
5-OMe	6-OMe	S	H	4-NO_2
6-OMe	7-OMe	S	H	4-NO_2
5-OMe	7-OMe	S	H	4-NO_2
4-OMe	H	S	2-Cl	4-NH_2
5-OMe	H	S	2-Cl	4-NH_2
6-OMe	H	S	2-Cl	4-NH_2
5-OH	H	S	2-Cl	4-NH_2
6-OH	H	S	2-Cl	4-NH_2
5-OBenzyl	H	S	2-Cl	4-NH_2
H	H	S	6-OSO_2CF_3	3-NH_2
H	H	S	3-OSO_2CF_3	4-NH_2
6-Me	H	S	3-OSO_2CF_3	4-NH_2
H	H	S	5-OSO_2CF_3	2-NH_2
7-OBenzyl	H	S	2-Cl	4-NH_2
6-OMe	H	S	H	4-NH_2
6-OH	H	S	H	4-NH_2
6-Me	H	S	H	4-NH_2
5-OMe	7-OMe	S	H	4-NH_2
5-OH	7-OH	S	H	4-NH_2
5-OH	7-OMe	S	H	4-NH_2
5-OMe	6-OMe	S	H	4-NH_2

In preferred compounds, as may be apparent from the

above list, R^1 and R^3 will often both be hydrogen but one or other may alternatively be alkoxy or aralkoxy, e.g. methoxy or benzyloxy, whilst at least one of R^2 and R^4 is preferably an amino or a substituted amino group (e.g. -NMe_2) but may alternatively be nitro, azido, hydroxy, halo, or R^2 and R^4 may collectively comprise a combination of such groups substituted at different positions. Other substitution patterns can also be of interest as will be apparent from examples herein disclosed. Although in some cases the nitro and azido substituted compounds themselves exhibit some selective cytotoxic activity, most commonly these derivatives will be used as intermediates for reduction to obtain the corresponding amino compounds. The latter are usually more likely to show a useful level of biological activity.

One especially important compound in the above list is that designated CJM 126 wherein R^1, R^2 and R^3 are each hydrogen, R^4 is 4-NH_2 and X is sulphur, i.e. 2-(4-amino-phenyl)benzothiazole, including pharmaceutically acceptable salts thereof.

Salts of the compounds of formula I in accordance with the invention may include acid addition salts derived from an acid selected from the group comprising: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, salicylic, p-toluenesulphonic, tartaric, citric, lactobionic, formic, malonic, pantothenic, succinic, naphthalene-2-sulphonic, benzenesulphonic, methanesulphonic and ethanesulphonic.

The invention also comprises the use of a 2-arylbenzazole compound as specified above for making a medicament or pharmaceutical composition for use in antitumour therapy, especially in the treatment of breast cancer for example.

As hereinafter described, the invention also includes pharmaceutical compositions or preparations, conveniently

in unit dosage form, for use in antitumour therapy, especially breast cancer therapy, said compositions or preparations comprising as the active substance a 2-arylbenzothiazole or 2-arylbenzoxazole compound as specified above.

In general, salts of compounds such as CJM 126, e.g. hydrochloride, dihydrochloride, methanesulphonic acid and ethanesulphonic acid addition salts, should be equally effective in inhibiting proliferation of breast tumour cells insofar as such salts will dissociate in water or other aqueous media to provide the active antitumour compound. In practice these salts may be the most preferred compounds for making up acceptable pharmaceutical formulations.

Development and Testing

The invention has developed from an initial observation made when carrying out certain routine chemical investigations. During these investigations it was found that the arylbenzothiazole compound 2-(4-aminophenyl)benzothiazole, herein designated CJM 126 and sometimes referred to as ptiazammine, had a very surprising and completely unexpected high and selective activity as an anti-proliferative agent in respect of cultures of MCF-7 mammary carcinoma cells.

This is illustrated in Tables 1 and 2 at the end of the present description which show in vitro test results obtained in various sets of experiments for the cytotoxic activity of this compound CJM 126 against a range of human tumour cell lines, expressed in terms of IC_{50} values (concentration or dosage required to reduce cell growth or proliferation by 50%) calculated from dose-effect curves plotted for cultures of the cells in question.

It will be seen from Table 1 that the MCF-7 cell line used in the first series of experiments was about 3000

times more sensitive to CJM 126 than other human cell lines tested. This remarkable result has been reconfirmed on several subsequent occasions, as evidenced for example by the results presented in Table 2, using fresh samples and different strains of the MCF-7 cells in later series of experiments. Thus, selective inhibition has been clearly demonstrated, for example against the following human mammary carcinoma cell lines: MCF 7-wt, MDA 468, SKBR3, ZR 75 and MDA 231 with estimated IC_{50} values of about 2.9nM, 1.6nM, 25.9nM, 24.7nM, and 17.2nM respectively. Also, a somewhat unusual biphasic dose response relationship was noted. For example, in the first series of experiments referred to above it was found that an initial decrease in cell growth for the MCF-7 cells, which gave the IC_{50} value of approximately 2 ng/ml, was followed at higher doses by a small increase, and then at even greater doses there was a second decrease corresponding to an IC_{50} value of about 2 µg/ml. This biphasic dose response effect has also been found to be consistently reproducible. Thus, in the later series of experiments 30nM and 100nM CJM 126 resulted in maximum growth inhibition of MCF-7 cells and loss of growth arrest, characterised by rising cell numbers, followed exposure to 1µM, 3µM and 10µM. For MCF 7-ADR cells at low concentrations of CJM 126 growth was negligible under the experimental conditions used (seeding density 2.5 x 10^2 cells per well). However, at higher concentrations of 3 and 10µM, CJM 126 induced proliferation of these MCF cells 7-ADR.

Although certain mammory carcinoma cell lines examined did not give a biphasic dose response following CJM 126 exposure, cell lines derived from tumours of other origins appeared generally to be insensitive to the specific inhibitory properties of CJM 126. No effect was observed, for example, on the growth of A431 cells as a result of exposure to CJM 126 over a range of concentrations (0.1nM-10µM). Estimated IC_{50} values for some of the other cell lines tested are given in Table 2.

In other in vitro studies it was found that CJM 126 gave an IC_{50} value of 2.7×10^{-5} M against P388 cells, and against P388R8/22 (multidrug resistant) cells it gave an IC_{50} value of 5.2×10^{-5} M. CJM 126 was also tested against the NCI (National Cancer Institute, U.S.A.) panel of human tumour cell lines which does not contain any breast cancer cell lines, and the compound was found to be essentially non-toxic in these tests.

In carrying out the above in vitro studies, the method used for conducting the cytotoxicity assays was generally substantially as follows:

Cells were maintained in a continuous logarithmic culture in Dulbecco's medium supplemented with 10% fetal calf serum and penicillin (100 IU/ml) and streptomycin (100 µg/ml). The cells were mildly trypsinized for passage and for use in assays. On day zero, 100µl of trypsinized tumour cells (1×10^4/ml) were plated in the wells of 96-well flat-bottom microtiter plates. The plates were incubated for 2 days at 37°C and 5% CO_2 in air to allow the cells to adhere and resume exponential growth prior to the addition of drugs.

The compounds being tested were dissolved in a small volume of DMSO and diluted to the desired concentration with growth medium so that the final concentration of DMSO did not exceed 0.25%. On day two 50µl of the highest drug concentration was added to the wells of column 12 and from there serially diluted 3-fold to column 1 by serial transfer of 50µl using an 8-channel micropipette. The final volume of column 1 was adjusted to 100µl. No additions were made to the wells of rows A and B, which served as controls. The plates were further incubated for 5 days at 37°C and 5% CO_2 in air. Each compound was tested in duplicate.

On day 7 the test was terminated by the addition of

100µl saline containing 0.002% w/v propidium iodide (Sigma), 0.3% drawing ink (Staedtler "Marsmatic 745" - Trade Mark) and 0.5% Triton X-100. The plates were kept at 4°C overnight before reading on an inverted microscope equipped with an automated scanning stage. Fluorescence intensity was measured in arbitrary units by a photomultiplier. An HP-87 computer controlled the movement of the stage and also collected and processed the data from the multiplier.

For each compound tested a dose-response curve was obtained and the IC_{50} value (the drug concentration at 50% inhibition of cell growth) was calculated.

The remarkably specific effects and activity of CJM 126 noted against human MCF-7 cells in vitro has been found subsequently to extend to in vivo tests and also to a broad range of analogues of CJM 126, as illustrated for example in Tables 3, 4 and 5.

Table 3 presents the results, including estimated IC_{50} values, of cytotoxicity and proliferation or growth stimulation tests carried out in vitro for different breast cancer cell lines, including MCF7-wt, MDA 468 and MCF 7-ADR, in respect of compound CJM 126 and a range of analogues of CJM 126 identified by reference codes in the first column. The structures of these analogues will be apparent from the subsequent description herein.

The results of some of the in vivo tests carried out, and a comparison of the activity of CJM 126 with that of Mitoxantrone, are set out in Table 4. The results of similar tests carried out to evaluate the activity of CJM 126, and also of a close analogue thereof designated CJM 129, against BO (T61) human mammary carcinomas implanted in NMRI-nu/nu female mice are shown in Table 4 together with results obtained using other antitumour drugs for comparison. The results marked * are all statistically significant ($p<0.05$). As will be seen, CJM 126 had

significant activity against MCF-7 and BO mammary tumours and there were no signs of severe toxicity following administration of a single i.p. injection of 120 mg/kg CJM 126 (Table 4). Additionally, the relative BO tumour volume was lower following daily treatment of 1mg/kg compared with 10 or 100mg/kg/day (Table 5).

The effect of CJM 126 (0.1, 0.01 and 0.001mg/kg) has also been investigated in nude mice bearing human MDA 468 xenograft tumours. The biphasic response obtained in vitro following exposure of MDA 468 cultures to CJM 126 was also observed in vivo when comparable CJM 126 doses were administered. Preliminary data indicate maximum inhibition to tumour growth following daily treatment of the minimum concentration tested, as can be seen from the summary of results presented in Table 6.

Although high concentrations of CJM 126 were found actually to stimulate cell proliferation in some cases, experiments have demonstrated that a continued exposure to CJM 126 is absolutely essential to maintain this stimulation of growth effect which is observed, for example, in the 2nd phase of the MCF 7-wt response. Removal of CJM 126 led not merely to loss of proliferative potential but also to a decline in cell numbers. In contrast, it was found that cell growth following exposure to nM CJM 126 concentrations (phase 1) remained arrested after drug removal and an observed IC_{50} value of 2.18nM was not significantly affected.

In these in vitro assays, it was noted that serum factors appear to play a significant role in the 2nd phase of the CJM 126 dose response. Thus, in the presence of 1% FCS, no proliferation of colonies was observed following exposure to 3, 10, 30μM CJM 126, and growth inhibition was maintained. Moreover, enhanced potency was detected with estimated IC_{50} values in the picomolar range.

In other experiments it was established that growth

of MCF 7-wt cell cultures supplemented with 1% FCS was stimulated 240%, 346% and 397% by 5, 50 and 100ng/ml EGF respectively, but CJM 126 was able to reverse this effect and exquisitely arrest the growth of these cells. However, the dose response profiles and IC_{50} values did not differ significantly from MCF 7-wt cultures supplemented by 1% FCS in the absence of EGF. It was also noted that the presence of EGF was unable to rescue the 2nd phase of the dose response, and inhibition of growth was greater than 85% for all concentrations of CJM 126 between and including 1nM-10μM. Similarly, in MCF 7-wt cultures supplemented with 10% FCS, the dose response to CJM 126 was not significantly altered by inclusion of EGF (5, 50 and 100ng/ml) in experimental media.

It was also observed that CJM 126 (1μM, 10μM and 100μM) showed no ability to displace iodinated EGF from EGFR. In addition, Western blot analyses utilizing an anti-phosphotyrosine primary antibody demonstrated no apparent inhibition of unstimulated or of EGF stimulated EGFR tyrosine kinase activity, and at present the mode of action of the CJM 126 compound in these biological systems is not known.

In the course of studying the in vivo activity it was found that the lethal dose value LD_{50} of CJM 126, i.e. the dose that was lethal to 50% of the animals tested, was about 125mg/kg in male DBA/2 mice when administered as a single i.p. dose. With daily administration over 3 consecutive days the LD_{50} dose was >31mg/kg/day.

Preparative Methods

In most cases the arylbenzazole compounds of the present invention can readily be synthesised by various routes from easily available starting materials, and by way of example, several such general synthetic routes, designated Route A, Route B, Route C, and Route D, are illustrated in Figure 1 of the accompanying drawings in relation specifically to

arylbenzothiazole compounds. A reduction scheme for converting a nitro substituent of an arylbenzothiazole compound into an amino substituent is also depicted as Route E. Such nitro compounds are often prepared for use as intermediates in producing the corresponding amino compounds which usually may be expected to possess greater cytotoxic activity.

Some of the arylbenzazole compounds of the present invention are also known compounds per se that are already commercially available.

In the general method for Route A, which is also applicable to the synthesis of corresponding benzoxazole compounds, typically a mixture of the 2-aminothiophenol (0.05 Mol.) (or the 2-aminophenyl) and the appropriate benzoic acid derivative (0.05 Mol.), together with polyphosphoric acid (85g), is heated at 190-230°C for 4 hours, cooled and poured into a mixture of 10% aqueous sodium bicarbonate (1000ml) and ice. The solid product may then be collected, washed with water and recrystallized.

In the general method for Route B, typically a mixture of 2-aminothiophenol (0.05 Mol.), the appropriate benzaldehyde (0.05 Mol.) and dimethylsulphoxide (30 ml) is heated to 180°C for 15 minutes, cooled and diluted with water (200 ml). The precipitate is then collected, washed with water and crystallised.

In the general method for Route C, assuming for example that R^2 is a nitro group NO_2, a solution of the 2-aminothiophenol (0.05 Mol.) in pyridine (50 ml) is added slowly to a mixture of the appropriate nitrobenzoyl chloride (0.05 Mol.) also in pyridine (50 ml) at 25°C. The reaction is exothermic and is cooled in an ice-bath. The mixture may then be diluted with water (200 ml) and the products are collected and washed with water.

In the general method for Route D, in a typical procedure

the appropriate substituted thiobenzanilide (1 Mol. equiv.) is finely powdered and mixed with a little ethanol to form a wet paste. A 30% w/v solution of aqueous sodium hydroxide (8 Mol. equiv.) is added and diluted with water to form a suspension/solution of the thiobenzanilide in 10% w/v aqueous sodium hydroxide. Aliquots of this suspension/solution are then introduced dropwise at one minute intervals into a stirred solution of potassium ferricyanide (4 Mol. equiv.) in water at 80-90°C. The reaction mixture is heated for a further 30 minutes, then cooled. The 2-arylbenzothiazole products are collected, washed with water and crystallised.

Where R^2 of the 2-arylbenzazole compound synthesised by any of the above routes (or by any other route) is a nitro group NO_2, this may generally be reduced and converted into the corresponding amine as follows (Route E):

A mixture of the 2-(nitrophenyl)benzazole compound in question (0.05 Mol,) and stannous chloride dihydrate (0.25 Mol.) in absolute ethanol (200 ml) is stirred and refluxed under nitrogen for 1 to 4 hours. The ethanol is then removed under reduced pressure and the residue is dissolved in ethyl acetate (4 x 100ml). The combined organic phases are next shaken with excess aqueous sodium hydroxide to liberate the free amine bases and dissolve the tin residues. The separated organic phase is washed with water, dried (magnesium sulphate) and the solvent is evaporated. Finally, the products are then crystallised.

EXAMPLES

The preparation of a number of particular compounds which are considered to be of interest for use as active therapeutic substances to inhibit proliferation of at least certain breast cancer cells and which provide examples of preferred embodiments of the invention, including CJM 126

and also analogues thereof, will now be described in more detail. The compound reference codes used in Table 3 are also quoted where applicable. It should be understood, however, that these specific examples are not intended to be construed in any way as a limitation in the scope of the invention.

Example I

2-(4-aminophenyl)benzothiazole (Compound CJM 126)

(a) In one particular example, 2-(4-aminophenyl)benzothiazole (Compound CJM 126) was prepared in 57% yield from 2-aminothiophenol and 4-aminobenzoic acid using synthetic Route A, the final product being crystallised from methanol as pale yellow needle crystals having a melting point 155-157°C (aqueous solubility about 3.8µg/ml).

(b) In another preparative example, the same compound 2-(4-aminophenyl)benzothiazole was prepared in 73% yield, as beige needles (m.p. 155-156°C) crystallised from methanol, by reduction with stannous chloride hydrate of 2-(4-nitrophenyl)benzothiazole. The latter was first obtained (as yellow crystals, m.p. 229-231°C, crystallised from methanol or dimethyl formamide) either via Route C (71% yield) from 2-aminothiophenol and 4-nitrobenzoyl chloride, or via Route D (10% yield) from the corresponding thiobenzanilide and potassium ferricyanide.

Example IA

Ethanesulphonic acid salt of 2-(4-aminophenyl)benzothiazole (Compound 93003)

To prepare the ethanesulphonic acid salt, 2-(4-aminophenyl)benzothiazole (0.66g) in ethyl acetate (100 ml) at 25°C was treated with ethanesulphonic acid (0.338g). The solid was collected and washed with ethyl acetate (3 x 50 ml) followed by diethyl ether (3 x 50 ml). The

ethanesulphonic acid salt (0.89g., yield 90%) had m.p. 211-213°C.

Example IB

Dihydrochloride salt of 2-(4-aminophenyl)benzothiazole

To prepare the dihydrochloride salt, 2-(4-aminophenyl)benzothiazole (1.5g) was dissolved in ethyl acetate (250 ml) and a stream of dry hydrogen chloride was passed through the solution for 20 minutes. The yellow dihydrochloride salt was collected and washed with ethyl acetate followed by ether. The dihydrochloride (1.61g) had a m.p. 267-269°C.

Example IC

2-(4-Aminophenyl)benzothiazole methanesulphonic acid salt

To a solution of 2-(4-aminophenyl)benzothiazole (0.5g, 2.21mmol) in ethyl acetate (65ml) was added dropwise methanesulphonic acid (0.215g, 2.21mmol) at room temperature. The reaction mixture was stirred for 30 minutes. The product was collected and washed with hot ethyl acetate to give a pale yellow powder (0.67g, 94%), m.p. 261-262°C; ν_{max}/cm^{-1} 3422, 2880, 2633, 1598, 1487, 1322, 1220, 1149, 1043, 967, 780, 755, 561; δ_H(DMSO-d_6) 8.10(1H, d, J7.9, 4-H), 8.00(3H, m, 7,2',6'-H), 7.52(1H, t, J7.3, 5-H), 7.42(1H, t, J7.5, 6-H), 7.06(2H, d, J8.5, 3',5'-H), 5.72(3H, br s, NH_3+), 2.41(3H, s, CH_3).

In vitro assay of this compound gave IC_{50} values of 0.0007μM against MCF 7-wt cells and 0.0024μM against MDA 468 cells, indicating a very high level of selective cytotoxic activity.

Example II

2-(2-Chloro-4-nitrophenyl)-4-methoxybenzothiazole

This compound was prepared from 2-chloro-4-nitro-2'-methoxythiobenzanilide according to the general procedure of synthetic Route D. A yellow solid formed was immediately collected by filtration when 2-chloro-4-nitro-2'-methoxythiobenzanilide was added to the solution of potassium ferricyanide. The crude product was recrystallised twice from methanol to give yellow crystals (46%), m.p. 187-188°C; ν_{max}/cm^{-1} 3432, 3097, 2836, 1570, 1521, 1476, 1385, 1347, 1277, 1262, 1041, 890, 773, 743; $\delta_H(CDCl_3)$ 8.60(1H, d, *J*8.8, 6'-H), 8.37(1H, d, *J*2.3, 3'-H),8.19(1H, dd, *J*2.3, 8.7, 5'-H), 7.55(1H, d, *J*8.1, 7-H), 7.43(1H, t, *J*8.1$_1$, 6-H), 6.97(1H, d, *J*7.9, 5-H).

Example III

5-Benzyloxy-2-(2-chloro-4-nitrophenyl)benzothiazole

This compound was prepared from 2-chloro-4-nitro-3'-benzyloxythiobenzanilide, again according to the general procedure of synthetic Route D. The crude reaction product was separated into three fractions by flash column chromatography on silica using EtOAc-hexane-chloroform (1:6:1) as the eluate. The first fraction (52%) was 5-benzyloxy-2-(2-chloro-4-nitrophenyl)benzothiazole, m.p. 156-157°C; ν_{max}/cm^{-1} 3449, 1603, 1521, 1342, 1277, 1181, 1055, 829, 737; δ_H ($CDCl_3$) 8.60(1H, d, *J*8.8, 6'-H), 8.45(1H, d, *J*2.2, 3'-H), 8.27(1H, dd, *J*2.3, 8.8 5'-H), 7.88(1H, d, *J*8.9, 7-H), 7.72(1H, d, *J*2.4, 4-H), 7.55-7.38(5H, m, Ph-H), 7.26(1H, dd, *J*2.4, 8.9, 6-H), 5.23(2H, s, CH_2O).

Example IV

5,7-Dimethoxy-2-(4-nitrophenyl)benzothiazole

This compound was prepared from 4-nitro-3',5'-dimethoxythiobenzanilide, also according to the general procedure of Route D. Recrystallisation from ethanol-EtOAc gave a yellow powder (60%), m.p. 238-239°C; ν_{max}/cm^{-1} 3442, 2949, 1605, 1579, 1528, 1428, 1352, 1312, 1155, 1126, 853, 820, 688; $\delta_H(CDCl_3)$ 8.37(2H, d, *J',5'-H), 8.26(2H, d, J*9.0, 2',6'-H), 7.24(1H, d, *J*2.0, 4-H), 6.58(1H, d, *J*2.0, 6-H), 4.01(3H, s, 7-OCH_3), 3.95(3H, s, 5-OCH_3); *m/z* 316(M^+), 270(M-OCH_3 and -CH_3).

Example V

6-Methoxy-2-(4-nitrophenyl)benzothiazole

This compound was prepared from 4'-methoxy-4-nitrobenzanilide, again according to the general procedure of Route D. The crude product was purified by flash column chromatography using EtOAc-hexane (1:3) as the eluate to give the title compound (62%), m.p. 216-217°C; ν_{max}/cm^{-1} 3448, 1593, 1518, 1342, 1315, 1265, 1219, 1066, 849; δ_H ($CDCl_3$) 8.34(2H, d, *J*9.1, 3',5'-H), 8.21(2H, d, *J*9.1, 2',6'-H), 8.00(1H, d, *J*9.0, 4-H), 7.38(1H, d, *J*2.5, 7-H), 7.15(1H, dd, *J*2.6, 9.0, 5-H), 1.58(3H, s, CH_3), m/z 289 (M^+), 271(M-CH_3).

Example VI

2-(4-amino-2-chlorophenyl)-4-methoxybenzothiazole (Compound 93005)

This amine was obtained in 77% yield by reduction of 2-(2-Chloro-4-nitrophenyl)-4-methoxybenzothiazole according to the general procedure of synthetic Route E. Characterisation of the product was as follows:
mp. 148-150°C; ν_{max}/cm^{-1} 3460, 3316, 3205, 2962, 1626,

1602, 1567, 1474, 1441, 1414, 1335, 1257, 1043, 772, 741; $\delta_H(CDCl_3)$ 8.19 (1H, d, J8.6, 6'-H), 7.49 (1H, d, J8.0, 7-H), 7.31 (1H, t, J8.0, 6-H), 6.91 (d, J7.8, 5-H), 6.76 (1H, d, J2.3, 3'-H), 6.64 (1H, dd, J2.3, 8.6, 5'-H), 4.07-4.04 (5H, m, OCH_3, NH_2).

Example VII

2-(4-Amino-2-chlorophenyl)-5-benzyloxybenzothiazole (Compound DF161)

This amine was obtained by reduction of 5-benzyloxy-2-(2-chloro-4-nitrophenyl)benzothiazole, again according to the general procedure of Route E. The crude product was chromatographed on silica using EtOAc-hexane (1:1) as the eluate to give 2-(4-amino-2-chlorophenyl)-5-benzyloxy-benzothiazole (90%), m.p. 119-121°C; ν_{max}/cm^{-1} 3456, 3380, 1629, 1599, 1443, 1260, 1171, 1051, 802; $\delta_H(CDCl_3)$ 8.12(1H, d, J8.6, 6'-H), 7.79(1H, d, J8.7, 7-H), 7.65(1H, d, J2.4, 4-H), 7.54-7.36(5H, m, Ph-H), 7.14(1H, dd, J2.5, 8.8, 6-H), 6.81(1H, J2.3, 3'-H), 6.69(1H, dd, J2.3, 8.6, 5'-H), 5.20(2H, s, CH_2O), 3.77(2H, s,NH_2).

Example VIII

2-(4-Aminophenyl)-5,7-dimethoxybenzothiazole (Compound DF1620)

This amine was formed by reduction of 5,7-dimethoxy-2-(4-nitrophenyl)benzothiazole, also according to the general procedure of Route E. The crude product was chromatographed on silica using EtOAc-hexane (1:1) as the eluate to give 2-(4-aminophenyl)-5,7-dimethoxybenzothiazole (89%), m.p. 150-152°C; ν_{max}/cm^{-1} 3417, 3326, 3203, 3000, 1599, 1580, 1485, 1415, 1219, 1150, 1124, 1035, 824, 807, 631; $\delta_H(CDCl_3)$ 7.91(2H, d, J8.6, 2',6'-H), 7.16(1H, d, J2.0, 4-H), 7.75(2H, d, J2.0, 6-H), 4.03(2H, s, NH_2), 3.96(3H, s, 7-OCH_3), 3.91(3H, s, 5-OCH_3).

Example IX

2-(4-Aminophenyl)-6-methoxybenzothiazole (Compound 93002)

This amine was formed by reduction of 6-methoxy-2-(4-nitrophenyl)benzothiaxzole, also according to the general procedure of Route E. The crude product was chromatographed on silica using EtOAc-hexane (1:1) as the eluate to give 2-(4-aminophenyl)-6-methoxybenzothiazole (92%), m.p. 191-193°C; ν_{max}/cm^{-1} 3454, 1626, 1605, 1465, 1436, 1264, 1222, 825; δ_H (DMSO-d_6) 7.79(1H, d, J8.9, 4-H), 7.70(2H, d, J8,6, 2',6'-H), 7.62(1H, d, J2.5, 7-H), 7.05(1H, dd, J2.6, 8.9, 5-H), 6.66(2H, d, J8.6, 3',5'-H), 5.84(2H, s, NH_2), 3.84(3H, s, OCH_3); m/z 256 (M^+), 241 (M-OCH_3).

Example X

2-(4-Amino-2-Chlorophenyl)benzothiazole (Compound 93004)

This amine was formed by reduction of 2-(2-chloro-4-nitrophenyl)benzothiazole, again according to the general procedure of Route E. Recrystallisation from methanol gave yellow crystals (93%), m.p. 92-94°C; ν_{max}/cm^{-1} 3455, 3299, 3194, 1630, 1620, 1432, 1312, 1262, 1050, 847, 757, 729, 699, 626; $\delta_H(CDCl_3)$ 8.10 (1H, d, J8.6, 6'-H), 8.04 (1H, d, J7.7, 4-H), 7.89 (1H, d, J7.9, 7-H), 7.47 (1H, dt, J1.3, 7.2, 5-H), 7.36 (1H, dt, J1.2, 7.3, 6-H), 6.78 (1H, d, J2.4, 3'-H), 6.66 (1H, dd, J2.4, 8.6, 5'-H), 4.02 (2H, s, NH_2).

Example XI

2-(4-Aminophenyl)-5,7-dihydroxybenzothiazole (Compound DF162Eb)

To a stirred solution of 2-(4-aminophenyl)-5,7-dimethoxybenzothiazole (0.24 g, 0.838 mmol), obtained from Example VIII, in dry dichloromethane (15ml) under nitrogen

and at -70°C, was added dropwise boron tribromide (1.0 M solution) in dichloromethane (5.9 ml) over 30 minutes. The mixture was kept stirred at -70°C for a further 1 hour, then allowed to warm slowly to room temperature and stirred overnight. The mixture was then again cooled to -70°C and methanol was added dropwise until no further reaction was observed. It was then poured into 8% (W/V) aqueous sodium hydroxide (50ml). After acidification with 5M hydrochloric acid to pH7, the mixture was extracted with dichloromethane-methanol (4:1) (3 x 80 ml). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was then separated into two fractions by flash column chromatography on silica using EtOAc-hexane (3:2) as the eluant. The second fraction (62%) was the required 2-(4-aminophenyl)-5,7-dihydroxybenzothiazole, m.p. 294°C (dec); ν_{max}/cm^{-1} 3438, 3342, 3216, 1603, 1482, 1456, 1434, 1295, 1159, 1093, 829; δ_H (DMSO-d_6) 10.30(1H, s, 7-OH), 9.43(1H, s, 5-OH), 7.70(2H, d, *J*8.6, 2',6'-H), 6.74(1H, d, *J*2.0, 4-H), 6.64(2H, d, *J*8.6, 3',5'-H), 6.34(1H, d, *J*2.0, 6-H), 5.84(2H, s, NH_2).

Example XII

2-(4-Aminophenyl)-6-hydroxybenzothiazole

This compound was obtained by demethylation with boron tribromide from 2-(4-aminophenyl)-6-methoxybenzothiazole (see Example IX) in a manner similar to that described above for Example XI. The crude product was chromatographed on silica using EtOAc-hexane (1:1) as the eluate to give the title compound (89%), m.p. 262-263°C; ν_{max}/cm^{-1} 3487, 3388, 1620, 1460, 1289, 1238, 1175, 830; δ_H (DMSO-d_6) 9.71(1H, s, OH), 7.72,7.66(3H, m, 4.2',6'-H), 7.32(1H, d, *J*2.3, 7-H), 6.91(1H, dd, *J*2.4, 8.7, 5-H), 6.65(2H, d, *J*8.6, 3',5'-H), 5.80(2H, s, NH_2); *m/z* 242 (M_+).

Example XIII

2-(4-Aminophenyl)benzoxazole (Compound DF140)

This provides an example of a benzaxazole instead of a benzothiazole. In accordance with synthetic Route A, a mixture of 2-aminophenol (1.5g, 0.0136mol) and 4-aminobenzoic acid (1.885g, 0.0136mol) in polyphosphoric acid (20g) was heated at about 190°C for 4 hours, then cooled and poured into 10% aqueous sodium bicarbonate (400 ml). The product was collected by filtration, washed with water and recrystallised from methanol-H_2O (10:1) to give small pale yellow crystals (1.76g, 62%), m.p. 176-178°C (lit 185°C); ν_{max}/cm^{-1} 3472, 3322, 3186, 1614, 1500, 1454, 1312, 1242, 1170, 1066, 830, 742; δ_H(DMSO-d_6) 7.87(2H, d, *J*8.6, 2', 6'-H), 7.69-7.64(2H, m, 5, 6-H), 7.36-7.28(2H, m, 4, 7-H), 6.70(2H, d, *J*8.6, 3', 5'-H), 6.02 (2H, s, NH_2).

Example XIV

2-(3-Amino-6-trifluoromethylsulphonyloxyphenyl)benzothiazole

2-(3-Azidophenyl)benzothiazole (1g) was added in small portions (5 x 0.2g) to a mixture of trifluoromethanesulphonic acid (4 ml), trifluoroacetic acid (5 ml) and trifluoromethylacetic anhydride (1 ml) at 0°C. After evolution of nitrogen ceased, the mixture was stirred at 20°C for 18 hours, basified with aqueous ammonia and the products extracted into ethyl acetate. The organic layer was washed with water, dried ($MgSO_4$) and evaporated to give a gum which was separated on silica with hexane-ethyl acetate (6:4) as eluent. The product obtained was the title compound (70% yield). (Found: M^+ 374. $C_{14}H_9F_3N_2O_3S_2$ requires MW 374).

An *in vitro* assay of this compound against MCF-7 (wild type) cells gave an IC_{50} value of 50nM which again indicates a useful level of activity.

Example XV

2-(4-Amino-3-trifluoromethylsulphonyloxyphenyl)benzothiazole

This compound was prepared in a similar way to the compound of Example XIV from 2-(4-azidophenyl)benzothiazole. The yield was 12%, (Found: M^+ 374. $C_{14}H_9F_3N_2O_3S_2$ requires MW 374).

Example XVI

2-(4-Amino-3-trifluoromethylsulphonyloxyphenyl)-6-methylbenzothiazole

This compound was prepared in a similar way to the compound of Example XIV from 2-(4-azidophenyl)-6-methylbenzothiazole. (Found: M^+ 388. $C_{15}H_{11}F_3N_2O_3S_2$ requires MW 386).

Example XVII

2-(2-Amino-5-trifluoromethylsulphonyloxyphenyl)benzothiazole

This compound was prepared in a similar way to the compound of Example XIV from 2-(2-azidophenyl)benzothiazole (Found: M^+ 374. $C_{14}H_9F_3N_2O_3S_2$ requires MW 374).

Other analogue compounds or derivatives of interest that have been prepared using Route A comprise

2-(4-Dimethylaminophenyl)benzothiazole
2-(4-Diethylaminophenyl)benzothiazole
2-(2-Aminophenyl)benzothiazole
2-(2-Fluorophenyl)benzothiazole
2-(4-Aminobenzyl)benzothiazole

and using Route B comprise

2-(4-Hydroxyphenyl)benzothiazole
2-(4-Pyridyl)benzothiazole
2-[4-(Pyrrolidin-1-yl)phenyl]benzothiazole (Compound 93006)

and using Route C comprise

2-(3-Nitrophenyl)benzothiazole
2-(2-Chloro-4-nitrophenyl)benzothiazole
4,4'-Bis(benzothiazol-2-yl)azobenzene

and using Route D comprise

2-(2-Chloro-4-nitrophenyl)-6-methoxybenzothiazole
2-(2-Chloro-4-nitrophenyl)-7-methoxybenzothiazole

and by reduction of the corresponding nitro compound using Route E comprise

2-(3-Aminophenyl)benzothiazole (Compound CJM 129)
2-(4-Amino-2-chlorophenyl)-5-methoxybenzothiazole
2-(4-Amino-2-chlorophenyl)-6-methoxybenzothiazole
2-(4-Amino-2-chlorophenyl)-7-methoxybenzothiazole

Further analogues or derivatives of interest that have been prepared include

2-(4-Azidophenyl)benzothiazole
2-[4-(Morpholin-4-yl)phenyl]benzothiazole (Compound 93008)
2-[4-(Piperidin-1-yl)phenyl]benzothiazole (93007)
1-(Benzothiazol-2-yl)-2-(4-dimethylaminophenyl)-ethene
4,4'-Bis(benzothiazol-2-yl)hydrazobenzene (Compound 126-126)
2,2'-Diamino-5,5'-Di-(benzothiazol-2-yl)biphenyl (Compound DF68D)

Commercially available analogue compounds that have been tested and considered to be of interest include 2-(4-aminophenyl)-6-methylbenzothiazole for which in vitro assays against MCF 7-wt cells gave an IC_{50} value of 0.38μM and against MDA 468 cells an IC_{50} value of 0.4μM.

It will be appreciated that some of the above listed compounds with the more complex molecular structures, for example DF68D and 126-126, may be expected to break down or to be metabolised after administration to a mammal so as to form a simpler 2-arylbenzazole compound that, in use, is the main biologically active component. Also, many of the compounds with nitrogen-containing substituents, such as azido groups and nitro groups, may similarly be converted, in use, within the body to a corresponding active amino compound.

Therapeutic Use

As already indicated, the compounds of this invention have been found to inhibit tumour cell proliferation and to have significant selective antitumour activity, especially in respect of breast cancers. Antitumour activity is evidenced for example by reduction of tumour cell number in mammals bearing breast cancer tumours and a consequent increase in survival time as compared to a control provided by animals which are untreated. Antitumour activity is further evidenced by measurable reduction in the size of solid tumours following treatment with the compounds of this invention compared to the tumours of untreated control animals.

Accordingly, as previously stated the compounds of the present invention are of particular interest for the treatment of breast cancer tumours, and the invention further provides a method for the treatment of a patient suffering from breast cancer. For this purpose, an effective non-toxic amount of the active 2-arylbenzazole compound, such as the 2-(4-aminophenyl)benzothiazole

compound or an acid addition salt or close analogue thereof as hereinbefore defined, may be suitably administered, orally, parenterally (including subcutaneously, intramuscularly and intravenously), or topically. The administration will generally be carried out repetitively at intervals, for example once or several times a day.

The amount of the active compound which is required in order to be effective as an antitumour agent for treating mammals will of course vary and is ultimately at the discretion of the medical or veterinary practitioner treating the mammal in each particular case. The factors to be considered by such practitioner, e.g. a physician, include the route of administration and pharmaceutical formulation; the mammal's body weight, surface area, age and general condition; and the chemical form of the compound to be administered. However, a suitable effective antitumour dose may be in the range of about 1.0 to about 75 mg/kg bodyweight, preferably in the range of about 5 to 40mg/kg with most suitable doses being for example in the range of 10 to 30mg/kg. In daily treatment for example, the total daily dose may be given as a single dose, multiple doses, e.g. two to six times per day, or by intravenous infusion for any selected duration. For example, for a 75kg mammal, the dose range could be about 75 to 500mg per day, and it is expected that a typical dose would commonly be about 100mg per day. If discrete multiple doses are indicated, treatment might typically be 50mg of the arylbenzazole compound as hereinbefore defined, given 4 times per day in the form of a tablet, capsule, liquid (e.g. syrup) or injection. On account of the biphasic dose response characteristics of many of these compounds, however, care should be taken, particularly in the initial stages of treatment, to ensure that dosage amounts are not too high.

While it may be possible for the active compound of this invention to be administered alone as the raw chemical, it is preferable to present the active compound

as a pharmaceutical formulation. Formulations of the present invention, for medical use, comprise the active compound together with one or more pharmaceutically acceptable carriers thereof and, optionally, any other therapeutic ingredients. The carrier(s) must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The present invention therefore further provides a pharmaceutical formulation comprising an arylbenzazole compound as hereinbefore specified (possibly in the form of a free base or a pharmaceutically acceptable acid addition salt) together with a pharmaceutically acceptable carrier thereof.

The formulations include those suitable for oral, rectal, topical and parenteral (including subcutaneous, intramuscular and intravenous) administration.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include generally the step of bringing the active compound into association with a carrier which constitutes one or more accessory ingredients. Usually, the formulations are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier or with a finely divided solid carrier or with both and then, if necessary, shaping the product into desired formulations.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, tablets or lozenges, each containing a predetermined amount of the active compound; as a powder or granules; or a suspension in an aqueous liquid or non-aqueous liquid such as a syrup, an elixir, an emulsion or a draught. The active compound may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the active compound in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Moulded tablets may be made by moulding, in a suitable machine, a mixture of the powdered active compound with any suitable carrier.

A syrup may be made by adding the active compound to a concentrated, aqueous solution of a sugar, for example sucrose, to which may be added any accessory ingredient. Such accessory ingredient(s) may include flavourings, an agent to retard crystallisation of the sugar or an agent to increase the solubility of any other ingredient, such as a polyhydric alcohol for example glycerol or sorbitol.

Formulations for rectal administration may be presented as a suppository with a usual carrier such as cocoa butter.

Formulations suitable for parental administration conveniently comprise a sterile aqueous preparation of the active compound which is preferably isotonic with the blood of the recipient.

In addition to the aforementioned ingredients, formulations of this invention, for example ointments, creams and the like, may include one or more accessory ingredient(s) selected from diluents, buffers, flavouring agents, binders, surface active agents, thickeners, lubricants, preservatives (including antioxidants) and the like.

From another aspect, the invention thus also comprises use of a benzazole compound as hereinbefore specified for the manufacture of a medical preparation for the treatment of breast cancer tumours.

Table 1. In vitro cytotoxicity of CJM 126 against human cell lines (1st series of experiments)

Cell Line		IC_{50}(ng/ml)
MCF-7	mammary carcinoma	2
A204	rhabdomyosarcoma	5943
T24	bladder carcinoma	16876
WiDr	colon carcinoma	6008
IGR37	melanoma	23986
HT29	colon carcinoma	7568
A2780	ovarian carcinoma	8826

Table 2. IC_{50} values for CJM 126 against human cell lines (2nd series of experiments)

Cell Line	$IC_{50}\mu M$
MCF 7 mammary (ER+)	0.003
MCF 7 mammary (ER+)	0.008
MCF 7-ADR mammary	stimulation > 1μM
ZR 75 mammary (ER+)	0.028
SKBR3 mammary (ER-)	0.026
MDA 468 mammary (ER-/EGFR+)	0.0016
MDA 231 mammary (EGFR+/erbB3+)	.017
T47D mammary (ER-)	44.1
MCF 7-B (ER+)	62.2
MCF 7-T (ER-)	12.87
A204 rhabdomyosarcoma	26
T24 bladder carcinoma	75
WiDr colon	26
IGR 37 melanoma	106
HT 29 colon	33
A2780 ovarian carcinoma	39
A2780 ovarian carcinoma	43
A2780/CisR	37
HX/62 ovarian carcinoma	120
SKOV-3 ovarian carcinoma	64
41M ovarian carcinoma	36
41M/CisP	40
CH 1 ovarian carcinoma	22
CH 1/CisR	33

Table 3 Cytotoxicity (IC_{50}) and growth stimulation of analogues of CJM 126 in breast cancer cell lines *in vitro*

Compound	MCF-wt (μM) A	MCF-wt (μM) B	MDA 468 (μM) A	MDA 468 (μM) B	MCF7-Adr (μM) A	MCF7-Adr (μM) B	MCF7-Adr (μM) C
CJM126	0.003	-	0.0016	-	-	-	>1
DF140	0.11	-	-	>10	-	-	10, 30
DF161	0.001	55.9	0.0007	2.84	-	84.8	3,10,30
93002	0.38	-	-	7.64	-	-	3,10
93003	0.0046	-	0.0024	-	-	-	3,10
93004	0.142	-	0.003	-	-	-	1,3,10
CJM129	-	>10	NT	NT	Inactive		
DF126-126	-	>10	NT	NT	Inactive		
93005	0.063	-	0.0022	NT	0.0465	-	3,10
93006	0.867	-	NT	NT	-	-	3,10
93007	-	2.77	NT	NT	-	-	1,3,10
93008	0.160	-	NT	NT	-	-	3,10
DF162Eb	-	49.65	-	16.3		13.9	-
DF162D	-	19.3	0.0035	-	-	20.9	-
DF68D	-	22.3	-	19.3	Inactive		

A IC_{50} at sub-micromolar concentrations
B IC_{50} at >micromolar concentrations
C Concentrations which stimulate cell growth
NT Not tested

Table 4 *In vivo* activity of CJM 126 against MCF-7 mammary carcinoma

Compound	Route	Dose mg/kg/day	BWC (%)	Relative tumour volume (T/C%) d9	d13	d20	d27	d35	d43
CJM 126	i.p.	120	-13	84	47*	38*	27*	27*	43*
Mitoxantrone	i.v.	10	-10	52	32*	38*	26*	29*	29*

Mice: Bln. NMRI - nu/nu (female)

Tumour: MCF-7 implanted s.c. supplemented with oestradiol

Treatment schecule: qd 6, 13, 20 by indicated route.

BWC: Body weight change relative to saline treated animals

Control: Saline

*Statistically significant ($p<0.05$)

Table 5 *In vivo* activity of CJM 126 and CJM 129 against BO mammary carcinoma

Compound	Route	Dose (mg/kg/day)	BWC	Relative tumour volume (T/C%) d33	d41	d47	d55	d61
CJM 126	i.p.	100	-7	137	77	87	68	52
CJM 126	i.p.	10	-4	107	74	55	70	52
CJM 126	i.p.	1	-5	173	72	25*	67	41*
CJM 129	i.p.	200	-6	78*	45*	32*	33*	36*
CJM 129	i.p.	20	-3	88	77	54	73	48*
CJM 129	i.p.	2	2	127	91	94	95	78
Vincristine	i.p.	1	-6	142	62	61	61	57
Cyclophosphamide	i.p.	150	-4	117	27*	29*	19*	5*
Mitoxantrone	i.v.	10	-5	58*	26*	6*	7*	4*

Treatment schedule : for CJM 126 and CJM 129 qd 27, 34, 41; for vincristine, cyclophosphamide and mitoxantrone qd 27.

CJM 129 is 2-(3-aminophenyl)benzothiazole

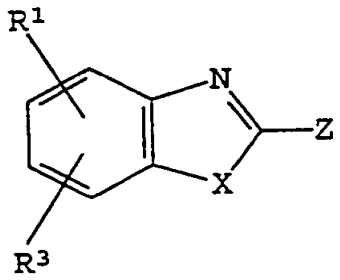
For other details: see footnotes to Table 4

Table 6. Activity of CJM 126 against human MDA 468 tumour *in vivo*.

	Cross section tumour area (mm) Group			
Day	Control	.001mg/kg	.01mg/kg	0.1mg/kg
5	95.28 + 4.61	85.54 + 19.44	120.78 + 5.83	97.75 +9.55
12	62.88 + 8.10	52.93 + 2.65	71.65 + 11.67	71.07 +7.2
15	86.02 + 8.65	67.81 +12.92	78.21 + 6.69	82.17 + 10.58
19	118.35 + 37.1	64.8 + 17.35	98.03 + 33.51	88.84 + 3.69
22	119.67 + 19.05	75.12 + 20.05	117.02 + 42.1	105.66 + 5.49
26	130.97 + 20.61	81.53 + 26.16	131.58 + 23.41	124.55 + 7.90
29	170.99 + 51.67	114.65 + 34.57	164.90 + 37.18	182.15 + 14.79
32	191.0 + 48.36	129.06 + 32.08	191.68 + 45.65	196.66 + 18.65
	Tumour weight (g)			
	.31 +.09	.19 + .08	.29 + .15	.30 + .07

CLAIMS

1. For use in therapy, a compound that is a benzazole compound represented by the structural formula I below, or a pharmaceutically acceptable salt thereof,



I

characterised in that

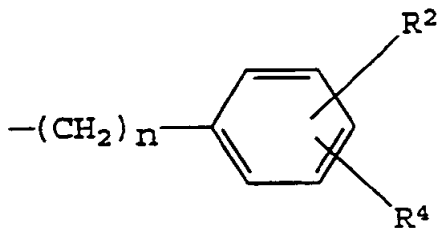
X is S or O;

R^1 and R^3 are each independently hydrogen, alkyl, hydroxyl, alkoxy or aralkoxy

Z is aryl;

subject to the proviso that alkyl groups when present as such in the compound or as a moiety in other groups such as alkoxy are each composed of less than 6 carbon atoms.

2. A 2-arylbenzazole compound as claimed in Claim 1 for use as a therapeutic agent wherein Z is



II

where n = 0 or 1,

R^2 is hydrogen, NH_2, NO_2, N_3, halogen or an alkyl or substituted alkyl oxysulphonyl group;

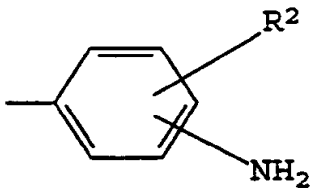
R^4 is hydrogen, NO_2, N_3, pyrrolidino, piperidino, morpholino or NR^5R^6 where

R^5 and R^6 each represent hydrogen or alkyl;

with the further proviso that R^2 and R^4 are not both hydrogen.

3. A compound as claimed in Claim 2 for use as an active therapeutic substance further characterised in that at least one of R^2 and R^4 is an amino or a substituted amino group.

4. A 2-arylbenzazole compound as claimed in Claim 2 for use as a therapeutic agent wherein Z is



5. A compound as claimed in Claim 4 for use as an active therapeutic substance wherein R^2 is 2-Cl.

6. A compound as claimed in any of Claims 2 to 5 for use as an active therapeutic substance wherein R^1 is hydrogen and R^3 is alkoxy or benzyloxy.

7. A compound as claimed in any of Claims 2 to 6 further characterised by at least one of the following features:

(a) at least some alkyl groups when present as such or as a moiety in other groups such as alkoxy are methyl or ethyl;

(b) halo substituents, when present, are selected from fluorine and chlorine;

(c) R^2 is hydrogen or 2-halogen, and R^4 is amino.

8. A 2-arylbenzazole compound as claimed in any of the

preceding claims for use as an active therapeutic substance, said compound being a substituted 2-phenylbenzothiazole compound.

9. A compound as claimed in Claim 2 for use as an active therapeutic substance, said compound being a substituted 2-phenylbenzazole compound characterised in that the combination of substituents R^1, R^2, R^3, R^4 and X is selected from the following combinations:

R^1	R^3	X	R^2	R^4
H	H	S	H	4-NH_2
H	H	S	3-NH_2	4-H
H	H	S	2-NH_2	4-H
H	H	S	H	4-NMe_2
H	H	S	H	4-NEt_2
H	H	S	H	4-Pyrrolidino
H	H	S	H	4-Piperidino
H	H	S	H	4-Morpholino
H	H	S	H	4-NO^2
H	H	S	3-NO_2	4-H
H	H	S	2-NO_2	4-H
H	H	S	H	4-N_3
H	H	S	3-N_3	4-H
H	H	S	2-N_3	4-H
H	H	S	3-I	4-NH_2
H	H	O	H	4-NH_2
H	H	O	H	4-N_3
H	H	S	2-F	4-NH_2
H	H	S	2-F	4-NO_2
H	H	S	2-F	4-N_3
H	H	S	2-Cl	4-NH_2
H	H	S	2-Cl	4-NO_2
H	H	S	2-Cl	4-N_3
4-OMe	H	S	2-Cl	4-NO_2
5-OMe	H	S	2-Cl	4-NO_2
6-OMe	H	S	H	4-NO_2
6-OMe	H	S	2-Cl	4-NO_2

	7-OMe	H	S	2-Cl	4-NO_2
	5-OBenzyl	H	S	2-Cl	4-NO_2
	6-OBenzyl	H	S	2-Cl	4-NO_2
	7-OBenzyl	H	S	2-Cl	4-NO_2
	5-OMe	6-OMe	S	H	4-NO_2
	6-OMe	7-OMe	S	H	4-NO_2
	5-OMe	7-OMe	S	H	4-NO_2
	4-OMe	H	S	2-Cl	4-NH_2
	5-OMe	H	S	2-Cl	4-NH_2
	6-OMe	H	S	2-Cl	4-NH_2
	5-OH	H	S	2-Cl	4-NH_2
	6-OH	H	S	2-Cl	4-NH_2
	5-OBenzyl	H	S	2-Cl	4-NH_2
	H	H	S	6-OSO_2CF_3	3-NH_2
	H	H	S	3-OSO_2CF_3	4-NH_2
	6-Me	H	S	3-OSO_2CF_3	4-NH_2
	H	H	S	5-OSO_2CF_3	2-NH_2
	7-OBenzyl	H	S	2-Cl	4-NH_2
	6-OMe	H	S	H	4-NH_2
	6-OH	H	S	H	4-NH_2
	6-Me	H	S	H	4-NH_2
	5-OMe	7-OMe	S	H	4-NH_2
	5-OH	7-OH	S	H	4-NH_2
	5-OH	7-OMe	S	H	4-NH_2
	5-OMe	6-OMe	S	H	4-NH_2

10. 2-(4-aminophenyl)benzothiazole for use as an active therapeutic substance or a pharmaceutically acceptable salt thereof.

11. A compound as claimed in Claim 1 for use as an active therapeutic substance, said compound being one of the following:

(1) 2-(4-Dimethylaminophenyl)benzothiazole
(2) 2-(4-Diethylaminophenyl)benzothiazole
(3) 2-(2-Aminophenyl)benzothiazole
(4) 2-(2-Fluorophenyl)benzothiazole
(5) 2-(4-Aminobenzyl)benzothiazole
(6) 2-(4-Hydroxyphenyl)benzothiazole

(7) 2-(4-Pyridyl)benzothiazole
(8) 2-[4-(Pyrrolidin-1-yl)phenyl]benzothiazole
(9) 2-(3-Nitrophenyl)benzothiazole
(10) 2-(2-Chloro-4-nitrophenyl)benzothiazole
(11) 6-Methoxy-2-(4-nitrophenyl)benzothiazole
(12) 2-(2-Chloro-4-nitrophenyl)-6-methoxybenzothiazole
(13) 2-(2-Chloro-4-nitrophenyl)-7-methoxybenzothiazole
(14) 2-(2-Chloro-4-nitrophenyl)-4-methoxybenzothiazole
(15) 2-(3-Aminophenyl)benzothiazole
(16) 2-(4-Amino-2-chlorophenyl)benzothiazole
(17) 2-(4-Amino-2-chlorophenyl)-4-methoxybenzothiazole
(18) 2-(4-Amino-2-chlorophenyl)-5-methoxybenzothiazole
(19) 2-(4-Amino-2-chlorophenyl)-6-methoxybenzothiazole
(20) 2-(4-Amino-2-chlorophenyl)-7-methoxybenzothiazole
(21) 2-(4-Azidophenyl)benzothiazole
(22) 2-[4-(Morpholin-4-yl)phenyl]benzothiazole
(23) 2-[4-(Piperidin-1-yl)phenyl]benzothiazole
(24) 4,4'-Bis(benzothiazol-2-yl)azobenzene
(25) 1-(Benzothiazol-2-yl)-2-(4-dimethylaminophenyl)-ethene
(26) 4,4'-Bis(Benzothiazol-2-yl)hydrazobenzene
(27) 2,2'-Diamino-5,5'-Di-(benzothiazol-2-yl)biphenyl
(28) Ethanesulphonic acid salt of 2-(4-aminophenyl)-benzothiazole
(29) Dihydrochloride salt of 2-(4-aminophenyl)benzo-thiazole
(30) 2-(4-Aminophenyl)benzothiazole methanesulphonic acid salt

12. A compound as claimed in any of the preceding claims for use as an active therapeutic substance characterised in that it is an acid addition salt derived from an acid selected from the group comprising: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, salicylic, p-toluenesulphonic, tartaric, citric, lactobionic, formic, malonic, pantothenic, succinic, naphthalene-2-sulphonic, benzenesulphonic, methanesulphonic and ethanesulphonic.

13. A process for preparation of a compound having the general structure defined in Claim 2 wherein R^2 and/or R^4 is amino, said process being characterised by the fact that it comprises the steps of preparing the corresponding nitro substituted compound, i.e. the compound with the same structural formula except that R^2 and/or R^4 is a nitro group, and then reducing said nitro group(s) to amino.

14. A compound as claimed in any one of Claims 1 to 12 for therapeutic use in treating breast cancer in mammals.

15. A pharmaceutical formulation for medical use comprising, as the active compound, a compound as claimed in any one of Claims 1 to 12 together with a pharmaceutically acceptable carrier therefor.

16. A medical preparation containing a therapeutically effective non-toxic amount of a compound as claimed in any one of Claims 1 to 12 and a pharmaceutically inert excipient.

17. A pharmaceutical preparation in unit dosage unit form for administration to obtain a therapeutic effect as an antitumour agent in treating mammals, said preparation comprising, per dosage unit, a therapeutically-effective non-toxic amount of a compound as set forth in any one of Claims 1 to 12.

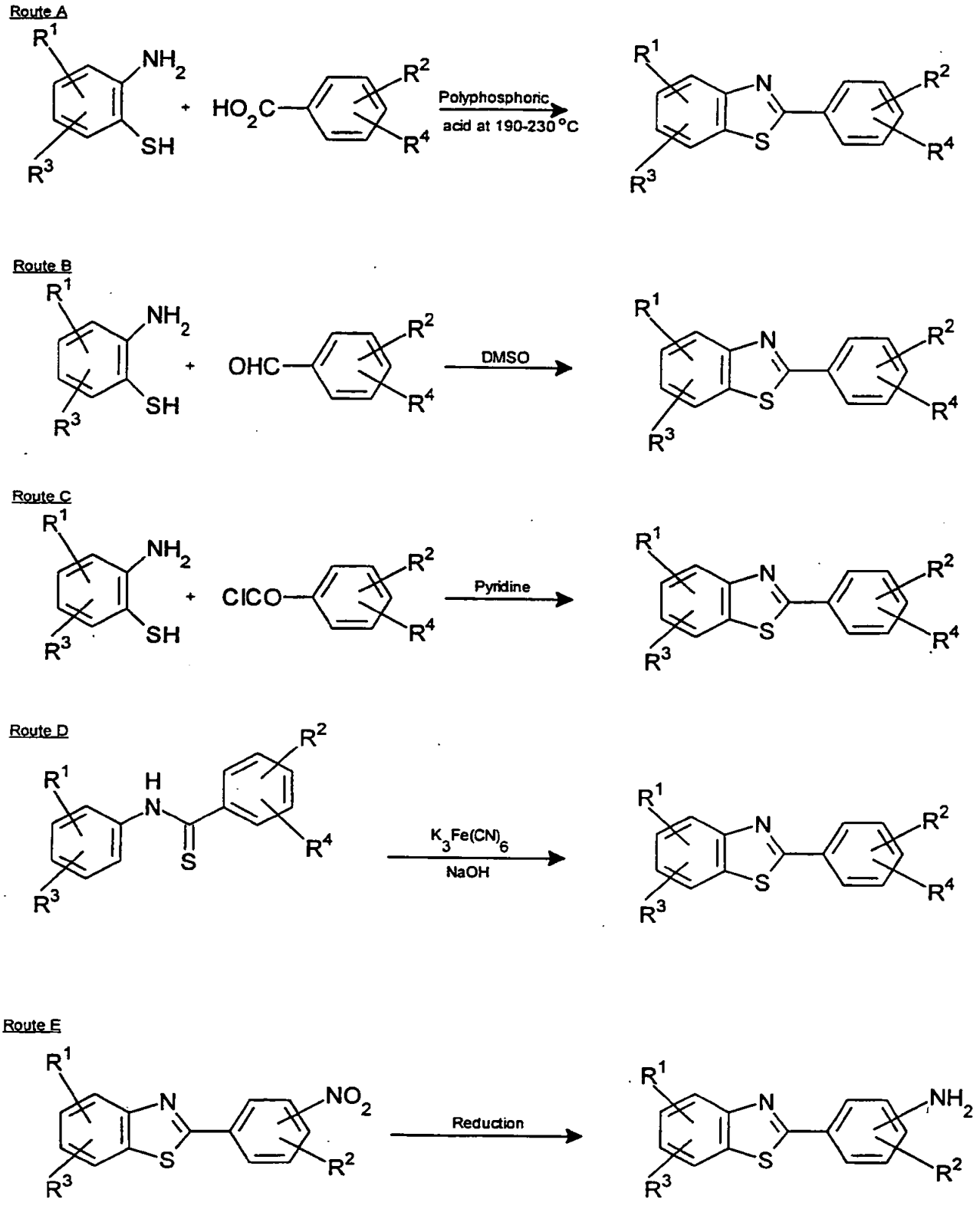
18. Use of a compound as claimed in any one of Claims 1 to 12 for the manufacture of a medical preparation for the treatment of tumours in mammals.

19. Use as claimed in Claim 18 wherein the medical preparation is for inhibiting the growth or proliferation of human breast cancer cells.

20. A method of treating a mammal suffering from cancer so as to inhibit or reduce cancer cell growth, especially growth or proliferation of breast cancer cells, said method

comprising administering to said mammal an effective antitumour composition wherein the active component is a benzazole compound as claimed in any one of Claims 1 to 12.

FIG. 1



INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 94/01883

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/425 C07D277/66 C07D263/56 A61K31/42

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 71, no. 5, 4 August 1969, Columbus, Ohio, US; abstract no. 22057s, W LEE ET AL 'Induction of increased benzopyrene hydroxylase activity by 2-phenylbenzothiazoles and related compounds' page 323 ; see abstract & CANCER RES., vol.28, no.12, 1968 pages 2539 - 2544 --- -/--	1-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 November 1994	21. 11. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Henry, J

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 113, no. 24, 10 December 1990, Columbus, Ohio, US; abstract no. 221786q, HU SHENGZHI ET AL 'Crystal structures and antitumor activity of 2-(2'pyridyl)benzothiazole and its organotin complex' page 661 ; see abstract & INORG. CHIM. ACTA, vol.173, no.1, 1990 pages 1 - 4 ---	1,14-20
X	PATENT ABSTRACTS OF JAPAN vol. 10, no. 208 (C-361) 22 July 1986 & JP,A,61 105 975 (TAKEDA CHEM. IND. LTD) 13 March 1986 see abstract ---	1-3,8, 14-20
X	PATENT ABSTRACTS OF JAPAN vol. 13, no. 402 (C-633) 6 September 1989 & JP,A,01 146 875 (KANEBO LTD) 8 June 1989 see abstract ---	1,2,8, 15,16
X	JOURNAL OF MEDICINAL CHEMISTRY, vol.13, no.5, September 1970, WASHINGTON US pages 1012 - 1013 STIG AKERFELDT 'Studies on the in vivo antiviral effects of benzothiazole derivatives against various influenza a2 srrains' see the whole document ---	1-4,15, 16
X	PATENT ABSTRACTS OF JAPAN vol. 6, no. 90 (C-104) 27 May 1982 & JP,A,57 021 375 (YAMANOUCHI PHARMCEUT.CO LTD) 4 February 1982 see abstract ---	1,2,8, 15,16
X	JOURNAL OF MEDICINAL CHEMISTRY, vol.13, no.4, July 1970, WASHINGTON US pages 697 - 704 THEODORE H. HASKELL ET AL 'Neuramidinase inhibition and viral chemotherapy' see the whole document ---	1,2,15, 16
X	US,A,2 780 628 (HERSCHEL D. PORTER) 5 February 1957	1-4, 9-11,15, 16
1	se column 6,lines 54,55 see the whole document ---	
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 94/01883

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB,A,2 164 648 (CIRD) 26 March 1986 see claims	1,2,15, 16
X	US,A,4 861 897 (JEFFERY B. PRESS ET AL) 29 August 1989 see column 2, line 39 - column 2, line 50; claims	1,2,15, 16
X	GB,A,1 093 355 (TWYFORD LABORATORIES LIMITED) 29 November 1967 see the whole document	1,2,15, 16
X	GB,A,1 080 246 (TWYFORD LABORATORIES LIMITED) 23 August 1967 see the whole document	1,2,15, 16
X	FR,A,1 255 312 (THE CROOKES LABORATORIES LIMITED) 30 January 1961 see the whole document	1,2,15, 16
X	FR,A,2 684 377 (SYNTHELABO) 4 June 1993 see claims	1,2,15, 16

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB94/01883

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 20 is directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the compounds

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
The wording Z=aryl is too broadly formulated to permit an adequate search. For this reason the search has essentially been restricted to compounds of formula I supported by the examples.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No
PCT/GB 94/01883

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-2780628		NONE		
GB-A-2164648	26-03-86	LU-A-	85544	03-04-86
		AT-B-	395714	25-02-93
		AU-B-	578310	20-10-88
		AU-A-	4755985	27-03-86
		AU-B-	593838	22-02-90
		AU-A-	6299586	08-01-87
		BE-A-	903254	18-03-86
		BE-A-	904421	30-06-86
		CA-A-	1256862	04-07-89
		CA-A-	1256804	04-07-89
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		CH-A-	675241	14-09-90
		DE-A-	3533308	27-03-86
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		NL-A-	8502557	16-04-86
		SE-B-	461466	19-02-90
		SE-A-	8504291	20-03-86
		SE-A-	8800949	16-03-88
		US-A-	4920140	24-04-90
		US-A-	5059621	22-10-91
		US-A-	5260295	09-11-93
		US-A-	4740519	26-04-88
		US-A-	5288744	22-02-94
US-A-4861897	29-08-89	NONE		
GB-A-1093355		NONE		
GB-A-1080246		NONE		
FR-A-1255312		NONE		
FR-A-2684377	04-06-93	NONE		